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ABSTRACT

Patients with high-risk localized prostate cancer have a high recurrence rate following primary therapy. Neoadjuvant chemotherapy has been shown to be beneficial in reducing recurrence rates in some tumor types, but has yet to be of proven benefit in prostate cancer. We utilized tissue resources from a phase II clinical trial of neoadjuvant chemotherapy with docetaxel and mitoxantrone in patients with high-risk localized prostate cancer to identify molecular alterations after chemotherapy, and correlated these alterations with clinical indicators of tumor response.

A chemotherapy-induced profile was generated by a direct comparison of expression changes between pre-treatment and post-treatment cancerous epithelia from prostate. After excluding genes previously shown to be influenced by the radical prostatectomy procedure, we identified 51 genes with significant transcript level alterations following chemotherapy. This group included several cytokines including GDF15, IL8, CXCL10, IL1B, and CCL2. *In vitro* analyses confirmed overexpression of GDF15 may confer resistance to chemotherapy in prostate cancer cells.

Gene expression changes after chemotherapy were further correlated with clinical outcomes including percentage of PSA decline and PSA-relapse free survival. Several chemokines and chemokine pathways were found to be associated with the percentage of PSA decline. Expression changes of IL8 and CXCL10 measured by qRT-PCR were significantly and negatively associated with the percentage of PSA decline. Further, *in vitro* tests showed only IL1B influenced chemosensitivity of prostate cancer cells.

When correlating expression profiles with PSA-relapse free survival, we found patients with a positive post-chemotherapy change in the expression of monoamine oxidase A (MAOA) have higher risk to have a PSA relapse compared with patients with negative post-chemotherapy MAOA expression change. *In vitro* studies confirmed the influence of MAOA on chemoresistance and determined that MAOA inhibitors produce additive effects on docetaxel-mediated prostate cancer cell growth inhibition.

In summary, genes and pathways that may contribute to chemotherapy resistance and response were identified. Development of small molecules or monoclonal antibodies capable of modifying cytokine activity, and utilizing existing drugs such as MAOA inhibitors may augment the effectiveness of chemotherapy response in patients with prostate cancer.

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INTRODUCTION:

Chemotherapy with docetaxel and/or mitoxantrone has been shown to be beneficial for some patients with advanced hormone refractory prostate cancer [1, 2]. However, there are no useful clinical and pathological markers to predict who will benefit from receiving these agents. In addition, the mechanisms used by tumor cells to circumvent the cytotoxic effects of chemotherapy are poorly understood, and thus cannot be effectively targeted to enhance tumor responses. Our hypothesis is that identifying *in vivo* gene expression changes before and after neoadjuvant chemotherapy will uncover the molecular mechanisms of prostate tumor response and resistance to cytotoxic drugs. Once identified, these tumor resistance mechanisms can be exploited through the design of combination therapies targeted toward inhibiting resistance pathways.

BODY:

Between January 2001 and November 2004, 57 patients with high-risk localized prostate cancer (defined as TNM > cT2b or T3a or PSA $\geq$ 15 ng/ml or Gleason grade $\geq$ 4+3) were recruited for a phase II trial clinical trial of neoadjuvant chemotherapy. The design of the clinical trial has been previously described [3, 4]. Figure 1 shows the schema of the study design. From each patient, ten standard prostate biopsies (bilateral at the apex, bilateral medial and lateral at mid-gland, bilateral medial and lateral at the base of the gland) were obtained under ultrasound guidance and snap-frozen in liquid nitrogen prior to chemotherapy. At the time of radical prostatectomy, cancer-containing tissue samples were snap frozen immediately after prostate removal. Evaluation of tissue samples identified the presence of adequate numbers of cancer cells in both pre-treatment and post-treatment samples for 31 subjects. We used laser capture microdissection techniques to specifically collect cancer epithelia from pre-treated biopsy specimens and post-treated radical prostatectomy specimens. Total RNA and cy3-cy5 labeled cDNA were generated based on the standard protocol in our lab. The strategy of hybridization is depicted in Figure 1.

Chemotherapy-Induced Profiles Reflect Differential Prostate Cancer Responses to Chemotherapy

A chemotherapy-induced profile was generated by a direct head-to-head hybridization between post-treated cancer epithelia and pre-treated cancer epithelia. Cancers surviving through docetaxel and mitoxantrone treatment are presumably enriched for resistant clones with molecular pathways contributing to cell survival. Hence, chemotherapy–induced profiles may reflect general mechanisms of chemotherapy resistance and response. After excluding 441 ischemia-related genes recognized in our previous study [5], we identified 53 genes with significantly alterations (p-value < 0.001) in post-treated specimens compared with pre-treated specimens by a random variance t-test (Table 1). Several altered genes encode cytokines such as IL-8, CCL2, GDF15, CXCL10 and IL1B. Cytokines may have important roles in modulating chemoresistance in cancer cells. A previous study has shown that IL-8, IL6, and CCL2 expression increased in paclitaxel-resistant ovarian cancer cell sublines [6]. GDF15 has been reported to be upregulated after neoadjuvant chemotherapy with epirubicin and cyclophosphamide or taxol in breast cancer [7]. These findings support our result using human tissues exposed to chemotherapy *in vivo*, and identified general pathways and gene expression changes that appear to be common across different cancers and/or chemotherapy agents.

The ultimate objective of this study is to identify chemotherapy resistance mechanisms that could be exploited as therapeutic targets to increase treatment responses. To this end, we further analyzed the functional categories of the 53 chemotherapy-altered genes based on GO biological processes using EASE software [8]. We found a significant enrichment of genes involved in cellular stress responses including categories of cell death and responses to abiotic stimulus, external stimulus, and chemical substances (Table 2). These findings are consistent with a cellular reaction to exogenous toxic agents. We also found a significant enrichment of genes involved in pathways of signal transduction, regulation

of transcription, cell communication, chemokine activity and inflammatory responses. These findings suggest that chemokines may play important roles in mediating chemotherapy resistance and response. Although chemokine activation after chemotherapy could be explained by generalized inflammatory reactions induced by cell death after chemotherapy, growing evidence indicates that chemokines are important survival factors for cancer cells under chemotherapy treatment [6, 9]. Chemokines and their receptors have the therapeutic advantage of modulation by agonists or antagonist such as small molecules or antibodies.

GDF15 Influences Chemotherapy Resistance

Cytokines have been shown to be associated with chemotherapy resistance and exert cytoprotective effects [6, 9]. Of the cytokine-encoding transcripts that we found to be differentially expressed, Growth Differentiation Factor 15 (GDF15), alias Macrophage Inhibitory Cytokine 1; a TGF-β superfamily member, has been reported to be associated with cancer progression and metastasis [10]. The role of GDF15 in chemotherapy resistance has yet to be determined. We first validated the expression results derived from the microarray analyses. GDF15 mRNA abundance of pre-treated biopsy and post-treated prostatectomy samples was measured by real-time PCR in aRNA obtained from the LCM material. Twenty-eight of 31 samples had measurable increases in GDF15 expression (90%) with 23/31 showing a 2-fold or greater change (Figure 2). The consistency of these findings suggests that GDF15 induction could be a generic response to chemotherapy stress, or could represent an important modulator of resistance in that there were no complete tumor responses in any of the subjects treated with 4 cycles of neoadjuvant therapy.

To explore these possibilities, we evaluated whether over-expression of GDF15 could confer cellular chemoresistance using an *in vitro* cell culture system. We over-expressed GDF15 by transfecting the DU145 cell with plasmids designed to express GDF15 and green fluorescent protein (GFP) (a kind gift from Dr. Breit [11]). DU145 cells overexpressing GDF15 and DU145 cells expressing only the GFP vector were treated with increasing concentrations of docetaxel or mitoxantrone for 3 days. The percentage of viable cells was determined using the MTS assay (Figure 3). We found that GDF15 expressing cells exhibited significantly greater resistance to both docetaxol and mitoxantrone-induced cytotoxicity, and the differences in cell survival were enhanced with increasing drug concentrations. To determine if GDF15 could exert a cytoprotective function via a paracrine mechanism, we treated parental DU145 cells with various concentrations of recombinant GDF15 protein and the chemotherapy drugs at around LD50 concentrations. We found that concentrations of GDF15 $\geq$1 ng/ml resulted in measurable increases in cell viability. Following 72 hours of exposure to docetaxel, 1 ng/ml GDF15 increased cell viability by 16% over no GDF15 treatment group, and 50 ng/ml increased viability by 52% (Figure 4A). A similar protective effect was also observed in cells treated with mitoxantrone (Figure 4B).

Expression Profiles Reflect Differential Prostate Cancer Responses to Chemotherapy

To investigate individual susceptibility to chemotherapy resistance and response, we need to correlate expression profiles with clinical outcomes such as PSA change after chemotherapy, PSA-relapse free survival, or pathological change after chemotherapy. However, detailed histopathological reviews of all radical prostatectomy samples in this study did not reveal any patient with a complete response, and partial pathological responses are difficult to accurately quantify, as the pre-treatment assessment of tumor volume is based on sampling by needle biopsies rather than having a complete organ for comparison. Thus, we used percentage of PSA decline after chemotherapy as an immediate clinical endpoint to explore possible association between chemotherapy induced profile and clinical outcome. PSA response is an immediate endpoint calculated from the serum PSA level measured before, during, and after chemotherapy. We have previously reported that the serum androgen levels were not

affected by the chemotherapy protocol employed here [4]. Thus PSA concentrations likely reflect changes in cancer cell numbers, though could potentially represent chemotherapy-mediated changes in cellular secretory mechanisms or tumor vasculature. Using SAM analysis (Significant Analysis of Microarray, http://www-stat.stanford.edu/~tibs/SAM/), we incorporated chemotherapy-induced gene expression profile and percentage of PSA decline after chemotherapy into a regression model. Percentage of PSA decline was defined as the maximal percentage decline of PSA level after chemotherapy compared with baseline PSA level before chemotherapy. The majority of patients had PSA declines after chemotherapy, however; six out of 31 patients had PSA elevations after chemotherapy (Figure 5). This might suggest inherent chemotherapy resistance among these 6 patients. SAM regression analysis showed that 26 upregulated genes associated with poorer chemotherapy response (FDR less than 25%). Only one downregulated gene associated with poorer chemotherapy response (Figure 5). Of these 27 genes, several associated with particular functional roles including: MAPK pathway (Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A and MAPK6), anti-apoptosis gene (Baculoviral IAP repeat-containing 2), transporter (Potassium voltage-gated channel subfamily H member 8, Solute carrier family 22 member 3) and the cytoskeleton. Gene pathway analysis showed 131 gene sets that associated with chemotherapy responses based on percentage of PSA decline. The top 10 gene sets included immune response (chemokine activity and receptor, JAK-STAT cascade, and response to bacterium), cell signaling (G-protein-coupled receptor binding and G-protein signaling coupled to cyclic nucleotide second messenger), musculoskeletal-associated genes (myosin light chain kinase activity and muscle development) and anti-apoptosis genes. As we have reported previously, several chemokines were not only induced by chemotherapy but were also shown to be associated with individual susceptibility to chemotherapy response (e.g. IL8). Expression of IL8 was significantly induced after chemotherapy. Furthermore, higher expression of IL8 associated with poorer chemotherapy response based on SAM regression analysis (Figure 5). We next measured gene expression changes among four chemokines including IL8, CCL2, CXCL10 and IL1B by quantitative real-time PCR (qRT-PCR). Expression changes measured by qRT-PCR were correlated with percentage of PSA decline by a univariate liner regression model. We found only IL8 and CXCL10 were significantly associated with percentage of PSA decline (Table 3 and Figure 6). Patients with higher expression of IL8 or CXCL10 seemed to be more resistant to chemotherapy with less percentage of PSA decline after chemotherapy.

IL1B may influence chemotherapy resistance

We further investigated the paracrine effect of IL8, CCL2, CXCL10 and IL1B on prostate cancer cells. The LNCAP cell line was chosen for further study because the baseline expression levels of these chemokines were extremely low by quantitative real-time PCR and Western blot. LNCAP cells were treated with various concentrations of recombinant IL8, CCL2, CXCL10 and IL1B (R&D Systems). We found IL1B increased percentage of viable cells by a MTS assay (Figure 7A) and decreased apoptosis activity by an apoptosis assay (Figure 7B) in LNCAP cells treated by docetaxel. The other three chemokines, IL8, CCL2 and CXCL10 did not influence chemoresistance of LNCAP cells based on *in vitro* cell culture tests.

Expression change of MAO-A may influence PSA free relapse

Percentage of PSA decline is an immediate clinical outcome, which may not completely reflect the longer-term prognosis of prostate cancer treated by chemotherapy. We further analyzed the chemotherapy induced profile against a third endpoint involving the determination of PSA-relapse free survival. For patients treated by radical prostatectomy, PSA serum levels are a good indicator of persistent or recurrent tumor when a threshold of 0.4 ng/ml and rising is used as an indicator of ultimate progression to metastasis [4]. We defined PSA relapse as a patient having two consecutive PSA

elevations greater than 0.4 ng/ml. There are 11 out of 31 patients having PSA relapse to date. A survival analysis method (http://linus.nci.nih.gov/BRB-ArrayTools.html) was used to profile differentially expressed genes associated with PSA-relapse free survival. Using default p-value of 0.001, we found 9 significant genes that were associated with PSA relapse-free survival including microtubule associated genes - tubulin-specific chaperone d and clathrin heavy polypeptide; membrane associate protein - metadeherin, transmembrane trafficking protein and pro-oncosis receptor inducing membrane injury gene and nardilysin; and three hypothetical proteins. Docetaxel directly acts on microtubule assembly and disassembly; and thus it is not surprising to see two microtubule associated genes associated with outcomes. In order to explore more candidate PSA relapse-free survival associated genes, we loosened the p-value to 0.01 and found 141 survival associated genes. Of the 141 genes, 101 were up-regulated and 40 genes were down-regulated in patients with PSA relapse. One of the survival associated genes; topoisomerase II alpha, was downregulated in patients with PSA relapse (Figure 8A), which is consistent with previous study showing chemosensitive testis cell lines had higher expression of TOP2A [12]. Another interesting gene; monoamine oxidase A (MAOA) was upregulated in patients with PSA relapse (Figure 8A). Quantitative real-time PCR (qRT-PCR) was performed to validate the MAOA expression change after chemotherapy. The expression change of MAOA by qRT-PCR was incorporated into a Cox Proportional Hazard Model using time to PSA relapse as the clinical outcome. Expression change of MAOA alone was statistically associated with PSA relapse-free survival by Cox model (hazardous ratio = 1.66, p-value= 0.027). After adjusting by Gleason sum score, expression change of MAOA still was marginally significant (hazardous ratio = 1.55, p-value= 0.068), which suggests that MAOA is an independent survival risk factor. We noticed that expression change of MAOA after chemotherapy was upregulated in patients with PSA relapse compared with patients without PSA relapse. We further investigated whether expression of MAOA may influence chemoresistance. We first tested whether chemotherapy exposure induces MAOA activity. Using MAO-Glo assay system (Promega), MAO activity was induced by docetaxel in LNCAP cells (Figure 8B). In order to see whether inhibition of MAOA activity will modify chemoresistance of prostate cancer cells, we treated LNCAP cell first with MAOA inhibitor (Clorgyline, Sigma) and then added docetaxel one hour after MAOA inhibitor administration. MTS cell proliferation assay was performed at 24 hours and 48 hours after docetaxel treatment. We found at 24 hours, MAOA inhibitor seemed to have an addictive effect (although not statistically significant) to low concentrations of docetaxel (10^{-9} and 10^{-8}M). The addictive effect was not as substantial at higher concentration of docetaxel (10^{-7}M, Figure 8C). At 48 hours, we still observed an addictive cell-inhibitory effect of MAOA inhibitor to low concentration docetaxel (Figure 8D). However, we did not observe any effect with mitoxantrone. The above results suggest MAOA expression may specifically influence chemoresistance of prostate cancer cells to docetaxel.

MAOA oxidizes neurotransmitters and dietary amines and influences the process of neurotransmitter metabolism as well as cell growth and differentiation. The byproduct of MAOA, aminoaldyhyde and hydrogen peroxide, may influence cell growth and differentiation [13]. Our previous study has shown prostate cancers with higher Gleason grade have higher MAOA protein expression [14]. Hence, MAOA may be related to tumorigenesis and/or tumor progression, but its roles in stress response and cell death are largely unknown. MAOA inhibitors may protect neuronal cells from apoptosis [15, 16]. Another study showed MAOA-inhibitors increased survival of non-tumorigenic keratinocyte treated by irradiation and cisplatin; however, MAOA inhibitor did not affect survival of HPV-16 transfected keratinocyte and PC3 cells and even decreased survival of a turmorigenic cell, ras-transfected keratinocyte [17], suggesting the effect of MAOA inhibitor on cell survival may be different between different tissue types and/or between normal and tumor cells. Our array findings suggest overexpression of MAOA after chemotherapy may confer resistance to chemotherapy and *in vitro* tests suggest inhibition of MAOA activity concurrently with docetaxel may further enhance cytotoxic effects.

KEY RESEARCH ACCOMPLISHMENTS:

- A chemotherapy-induced profile was identified. One of the cytokines, GDF15 may confer chemoresistance of prostate cancer cells to docetaxel and mitoxantrone by *in vitro* cell culture assays.
- We have identified alterations of gene expression associated with percentage of PSA decline after chemotherapy. Of the changes observed, the elevated expressions of several chemokines were specifically associated with PSA decline. Elevation of IL1B protein increased chemoresistance of LNCAP cells to docetaxel by *in vitro* cell culture assays.
- We have found gene expression changes associated with PSA-relapse free survival. Of these genes, we found patients with PSA relapse had higher expression of MAOA based on microarray and qRT-PCR analysis. *In vitro* cell culture systems suggested that inhibition of MAOA activity may have additive effects with docetaxel to inhibit prostate cancer cell growth.

REPORTABLE OUTCOMES:

Chung-Ying Huang, TomaszM. Beer, Celestia S. Higano, Lawrence D. True, Robert Vessella,Paul H. Lange, Mark Garzotto, and Peter S. Nelson. MolecularAlterations in Prostate Carcinomas that Associate with *In vivo* Exposure to Chemotherapy: Identification of a Cytoprotective Mechanism Involving Growth Differentiation Factor 15. *Clin Cancer Res. 2007 Oct 1;13(19):5825-33.*

Mengchu Wu, Chung-Ying Huang, Hong Gee Sim, Celestia S. Higano, Lawrence D. True, Robert Vessella, Paul H. Lange, Mark Garzotto, and Tomasz M. Beer Peter S. Nelson. Chemotherapy-Induced Monoamine Oxidase Expression in Prostate Carcinoma Associates with Clinical Outcome and Functions as a Cytoprotective Resistance Enzyme. Manuscript in preparation.

CONCLUSION:

In summary, we have identified candidate genes and pathways that may contribute to the response and resistance of prostate cancers to chemotherapy by generating and analyzing cellular gene expression profiles in the context of clinical outcomes. These results suggest that the development of small molecules or monoclonal antibodies capable of modifying chemokine expression, and utilizing existing drugs such as MAOA inhibitors may augment the effectiveness of chemotherapy responses in patients with prostate cancer. Through manipulating chemoresistance mechanisms and pathways, we may improve the outcomes of patients treated with cytotoxic chemotherapy for advanced disease.

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APPENDICES:

Table 1. Differentially expressed genes in post-treated samples compared with pre-treated samples.

Unigene	HUGO	Description	Ratio	p-value
Hs.624	IL8	Interleukin 8	3.27	< 1e-07
Hs.144513	TMEFF2	Transmembrane protein with EGF-like and two follistatin-like domains 2	2.39	0.00054
Hs.303649	CCL2	Chemokine (C-C motif) ligand 2	2.18	0.00010
Hs.466871	PLAUR	Plasminogen activator urokinase receptor	2.05	0.00000
Hs.413924	CXCL10	Chemokine (C-X-C motif) ligand 10	1.81	0.00070
Hs.504609	ID1	Inhibitor of DNA binding 1 dominant negative helix-loop-helix protein	1.80	0.00021
Hs.293736	ADNP	Activity-dependent neuroprotector	1.74	0.00000
Hs.515258	GDF15	Growth differentiation factor 15	1.71	0.00006
Hs.502829	SF1	Splicing factor 1	1.61	0.00001
Hs.76884	ID3	Inhibitor of DNA binding 3 dominant negative helix-loop-helix protein	1.56	0.00062
Hs.76753	ENG	Endoglin (Osler-Rendu-Weber syndrome 1)	1.50	0.00053
Hs.472651	BLCAP	Bladder cancer associated protein	1.49	0.00007
Hs.2178	HIST2H2BE	Histone 2 H2be	1.47	0.00052
Hs.244139	TNFRSF6	Tumor necrosis factor receptor superfamily member 6	1.46	0.00041
Hs.2030	THBD	Thrombomodulin	1.45	0.00001
Hs.270055	SH3GL3	SH3-domain GRB2-like 3	1.44	0.00048
Hs.126256	IL1B	Interleukin 1 beta	1.43	0.00035
Hs.549393	FOSL2	FOS-like antigen 2	1.41	0.00054
Hs.520140	SRF	Serum response factor	1.38	0.00055
Hs.470943	STAT1	Signal transducer and activator of transcription 1 91kDa	1.37	0.00056
Hs.159161	ARHGDIA	Rho GDP dissociation inhibitor (GDI) alpha	1.35	0.00014
Hs.435490	TMPRSS7	Transmembrane protease, serine 7	1.34	0.00013
Hs.59332	SPRED2	Sprouty-related EVH1 domain containing 2	1.34	0.00010
Hs.405662	CRABP2	Cellular retinoic acid binding protein 2	1.33	0.00004
Hs.516490	TANK	TRAF family member-associated NFKB activator	1.33	0.00001
Hs.429581	RTN4	Reticulon 4	1.32	0.00027
Hs.380906	MYADM	Myeloid-associated differentiation marker	1.30	0.00090
Hs.501309	CIRBP	Cold inducible RNA binding protein	1.29	0.00007
Hs.512908	ARPP-19	Cyclic AMP phosphoprotein 19 kD	1.27	0.00022
Hs.495960	ATP6AP2	ATPase H+ transporting lysosomal accessory protein 2	1.26	0.00084
Hs.487325	PRKACB	Protein kinase cAMP-dependent catalytic beta	1.26	0.00085
Hs.493096	PBX1	Pre-B-cell leukemia transcription factor 1	1.26	0.00071
Hs.370725	OSBPL1A	Oxysterol binding protein-like 1A	1.23	0.00087
Hs.73799	GNAI3	Guanine nucleotide binding protein alpha inhibiting activity polypeptide 3	1.22	0.00078
Hs.534312	TOR1A	Torsin family 1 member A (torsin A)	1.19	0.00066
Hs.444600	LAT1-3TM	LAT1-3TM protein	1.18	0.00055
Hs.517948	DHX30	DEAH (Asp-Glu-Ala-His) box polypeptide 30	1.18	0.00033
Hs.480073	HNRPD	Heterogeneous nuclear ribonucleoprotein D	1.16	0.00098
Hs.459779	DNAJA3	DnaJ (Hsp40) homolog subfamily A member 3	0.86	0.00061
Hs.248785	AGPAT3	1-acylglycerol-3-phosphate O-acyltransferase 3	0.84	0.00076
Hs.272062	PTPRF	Protein tyrosine phosphatase receptor type F	0.84	0.00075
Hs.433702	EIF5	Eukaryotic translation initiation factor 5	0.83	0.00041
Hs.302977	C12orf4	Chromosome 12 open reading frame 4	0.82	0.00088
Hs.25669	NCOA5	Nuclear receptor coactivator 5	0.79	0.00013
Hs.356247	ACY1L2	Aminoacylase 1-like 2	0.79	0.00018
Hs.301277	KIAA0543	KIAA0543 protein	0.79	0.00033
Hs.524183	FKBP4	FK506 binding protein 4 59kDa	0.76	0.00027
Hs.32417	SARG	Specifically androgen-regulated protein	0.75	0.00003
Hs.438545	SLC2A9	Solute carrier family 2 (facilitated glucose transporter), member 9	0.73	0.00004
Hs.411490	FAM36A	Family with sequence similarity 36 member A	0.72	0.00003
Hs.284491	PDXK	Pyridoxal (pyridoxine vitamin B6) kinase	0.68	0.00001
Hs.302738	SLC26A2	Solute carrier family 26 (sulfate transporter) member 2	0.63	0.00018
Hs.533977	TXNIP	Thioredoxin interacting protein	0.57	0.00004

Table 2. EASE functional categories of differentially expressed gene between pre- vs post-treated samples

GO biological process	EASE score	Gene Symbol
Signal transduction	0.004	ARHGDIA; CCL2; CRABP2; CXCL10; GNAI3; IL1B; IL8; OSBPL1A; PLAUR; PRKACB; PTPRF; SH3GL3; SRF; STAT1; TANK; TNFRSF6; TXNIP
Regulation of transcription	0.007	ADNP; CIRBP; CRABP2; FOSL2; HIST2H2BE; HNRPD; ID1; KIAA0543; NCOA5; PBX1; SF1; SRF; STAT1
Cell communication	0.010	ARHGDIA; CCL2; CRABP2; CXCL10; ENG; GNAI3; IL1B; IL8; OSBPL1A; PLAUR; PRKACB; PTPRF; SH3GL3; SRF; STAT1; TANK; TNFRSF6; TXNIP
Chemokine activity	0.019	CCL2; CXCL10; IL8
Response to abiotic stimulus	0.021	CCL2; CIRBP; CXCL10; IL8; OSBPL1A; PLAUR
Response to external stimulus	0.029	CCL2; CIRBP; CXCL10; IL1B; IL8; OSBPL1A; PLAUR; STAT1; TNFRSF6; TXNIP
Response to chemical substance	0.040	CCL2; CXCL10; IL8; PLAUR
Cell death	0.043	CCL2; FOSL2; IL1B; RTN4; STAT1; TNFRSF6
Inflammatory response	0.046	CCL2; CXCL10; IL1B; IL8

Table 3. Univariate linear regression analysis of expression changes of chemokine and percentage of PSA decline.

Chemokines	Regression Coefficient	P-value	95% CI
IL8	**-3.01**	**0.02**	**-5.55 ~ -0.47**
IL1B	-1.20	0.52	-4.94 ~ 2.55
CXCL10	**-5.86**	**0.02**	**-10.70 ~ -1.03**
CCL2	1.26	0.49	-2.40 ~ 4.93

Figure 1. Study design and hybridization strategy

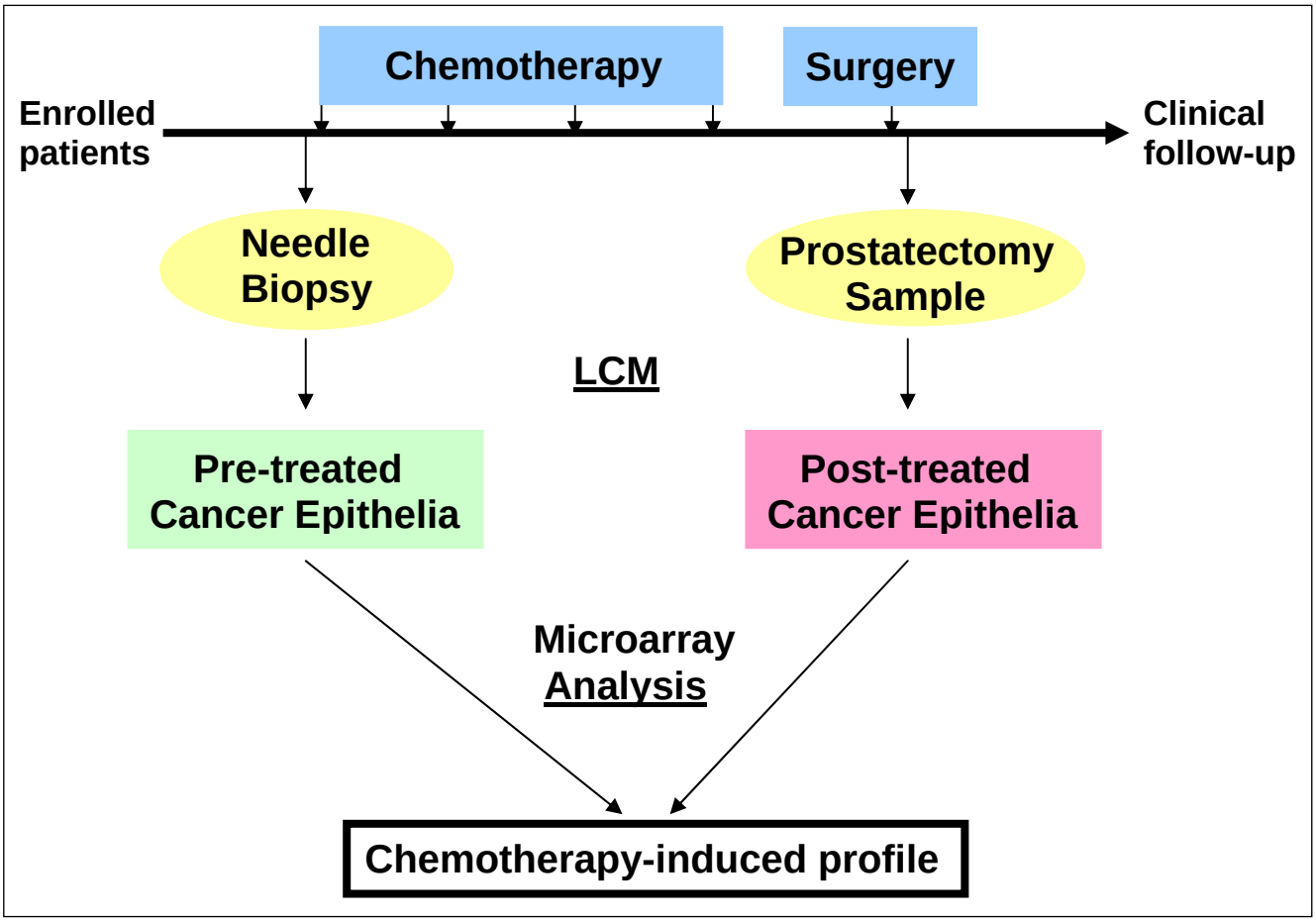


Figure 2. GDF15 expression in post-chemotherapy radical prostatectomy specimens compared with pre-chemotherapy needle biopsy specimens as determined by qRT-PCR. -△△CT was calculated as the differences of cycle threshold value (CT) of GDF15 in post-chemotherapy relative to pre-chemotherapy specimens. CT values of GDF15 were first normalized to the CT values obtained during the exponential amplification of GAPDH. Error bars represent one standard deviation for each sample. Dashed line indicates 2 fold differences.

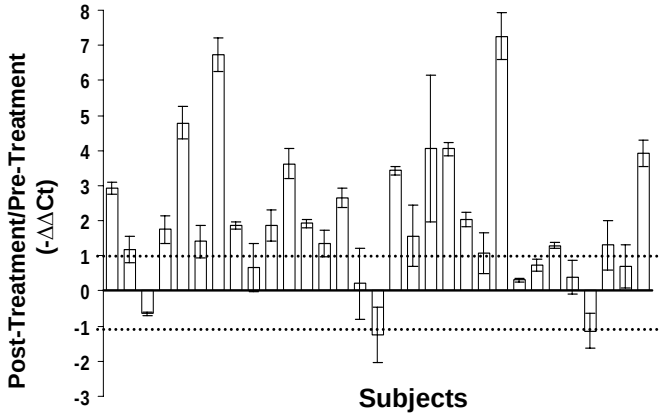


Figure 3. Influence of GDF15 expression on cell growth and chemotherapy resistance. DU145 (A,B) and PC3 (C,D) prostate cancer cells transfected with GDF15 expression vectors (solid line) versus empty (pIRES2) vector (dashed line) were exposed to docetaxel (A,C) or mitoxantrone (B,D) chemotherapy or vehicle control and quantitated by MTS assays. The y-axis indicates the cell numbers relative to vehicle control after 72 hours of treatment. The x-axis indicates concentration of docetaxel and mitoxantrone treatment. * p-value < 0.05 by student *t*-test compared to cells with empty vector.

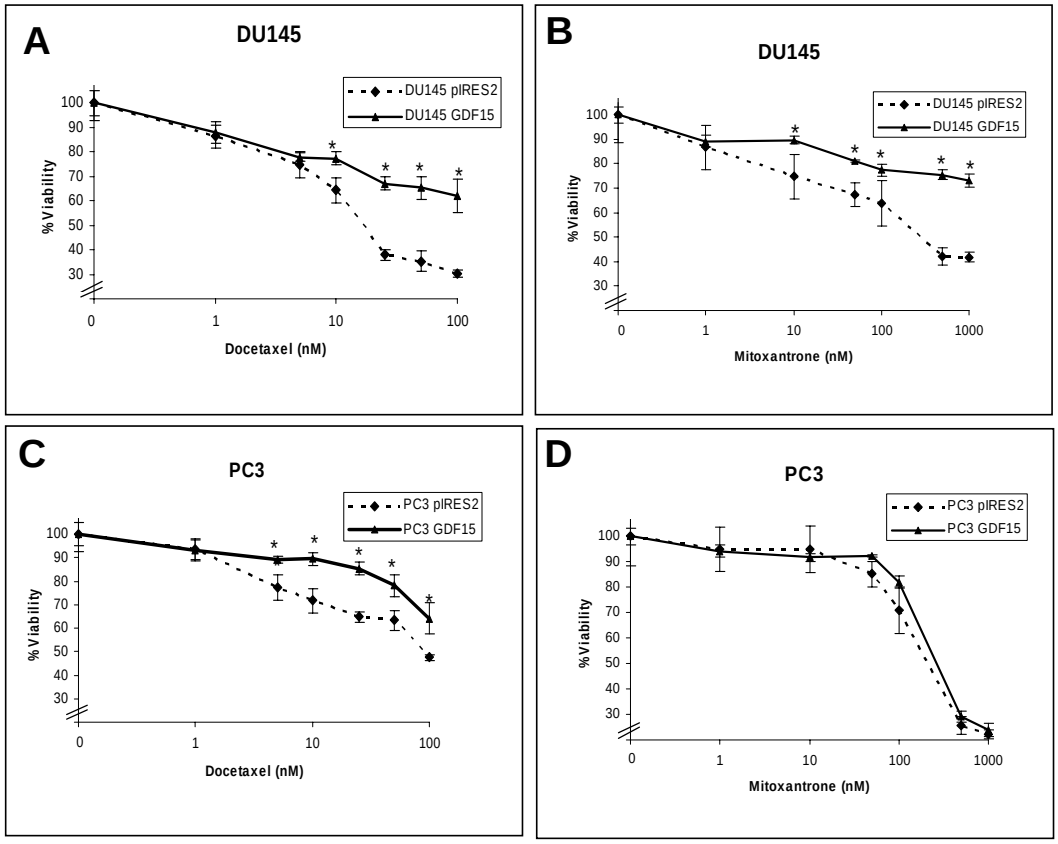
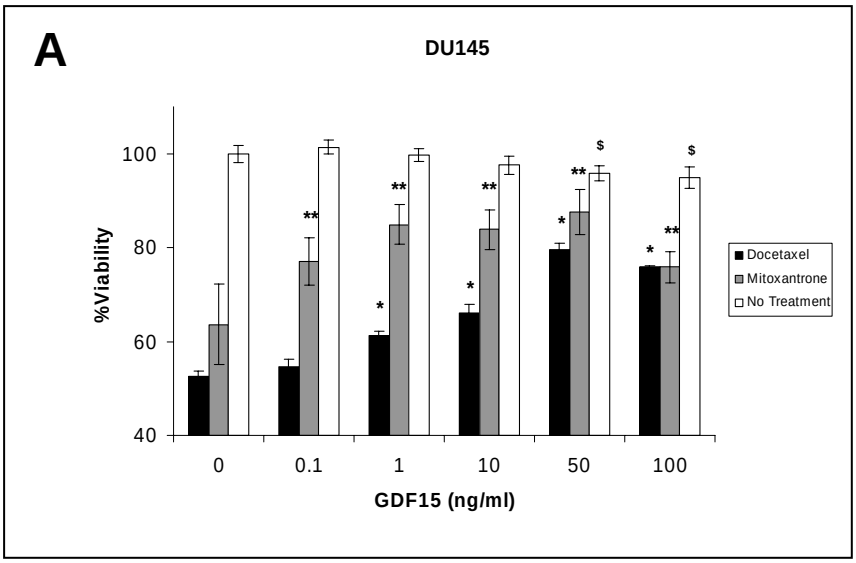


Figure 4. Influence of exogenous GDF15 on cell growth and chemotherapy resistance. MTS cell viability assay for prostate cancer cells treated with recombinant GDF15 protein. (A) DU145 cells. (B) PC3 cells. The concentration of docetaxel was 50 nM and mitoxantrone was 100 nM. The y-axis indicates the percentage of cells surviving after 72 hours of chemotherapy exposure relative to vehicle controls. The x-axis indicates the concentrations of GDF15 in growth media. * p-value < 0.05 by student *t*-test compared to no GDF15 treatment in the docetaxel treatment group (black bars). ** p-value < 0.05 by student *t*-test compared to no GDF15 treatment in mitoxantrone treatment group (shaded bars). $^{\$}$ p-value < 0.05 by student *t*-test compared to no GDF15 treatment in control (vehicle) group (white bars).



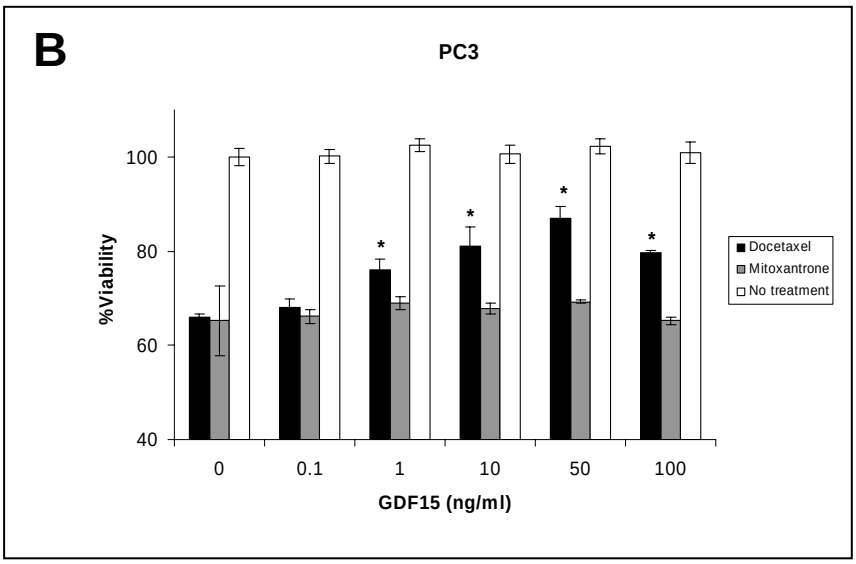


Figure 5. Differentially expressed genes associated with percentage of PSA decline after chemotherapy. (FDR < 25%)

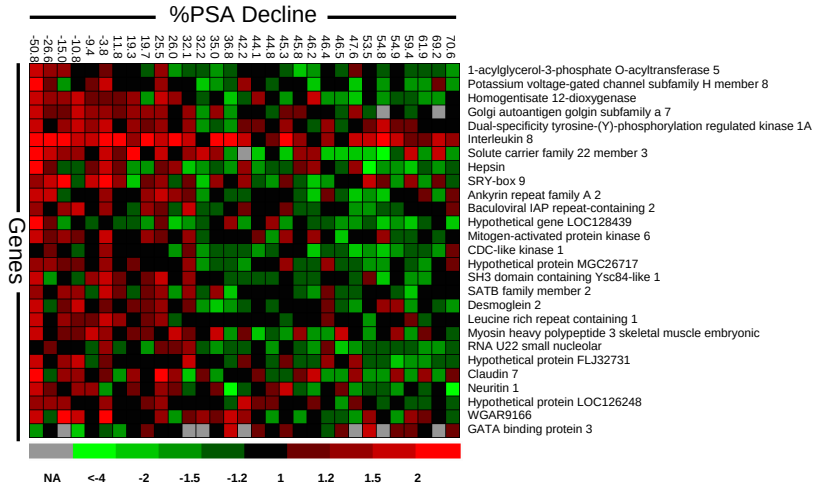
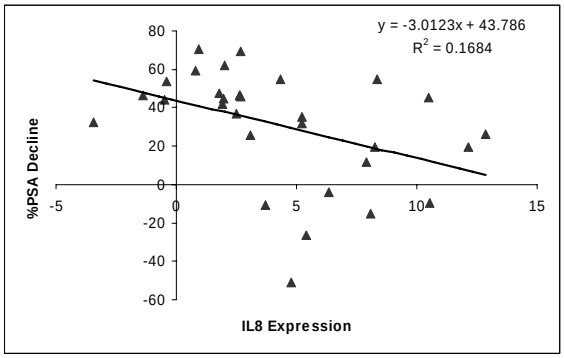
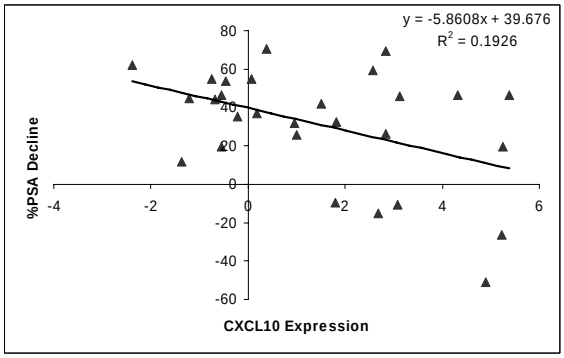
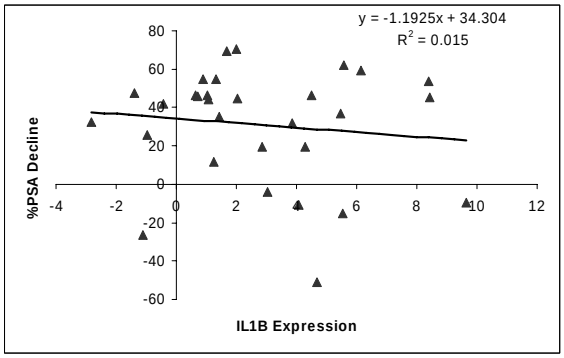


Figure 6. Linear regression of %PSA decline and expression changes of chemokines after chemotherapy. (X-axis: Cycle threshold difference between pre-treatment and post-treatment samples; Y-axis: percentage of PSA decline)







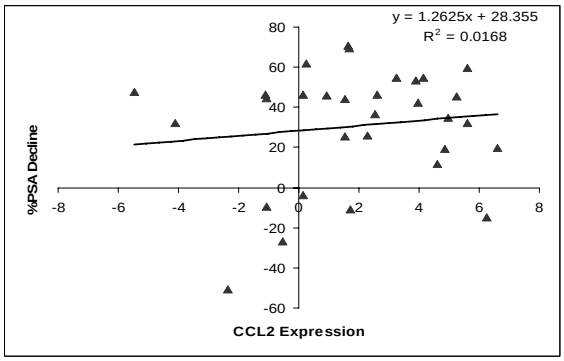


Figure 7. (A) Influence of exogenous IL1B on cell growth and chemotherapy resistance. Cell viability was measured by MTS assays of LNCAP cells treated with docetaxel and a series of recombinant IL1B concentrations. The concentration of docetaxel was 50 nM. White bars represent cells treated with IL1B and no docetaxel. Gray bars represent cells treated with 50 nM docetaxel and a series of IL1B concentrations. The y-axis indicates the percentage of cells surviving after 72 hours of chemotherapy and IL1B exposure relative to no IL1B treated control. The x-axis indicates the concentrations of IL1B in the growth media. * p-value < 0.05 by Student *t*-test when compared to no IL1B treated control. ** Error bar is one standard deviation. (B) Influence of exogenous IL1B on cell apoptosis and chemotherapy resistance. Cell apoptosis was measured by caspase 3/7 activity assay after 72 hours of docetaxel treatment. LNCAP cells were treated with 50 nM of docetaxel and a series of IL1B protein concentrations. White bars represent cells treated with IL1B without docetaxel. Gray bars represent cells treated with IL1B and 50 nM of docetaxel. The y-axis indicates caspase 3/7 activity. The x-axis indicates the concentrations of IL1B in growth media. * p-value < 0.05 by Student *t*-test when compared to docetaxel treatment without IL1B. ** Error bar is one standard deviation.

(A) (B)

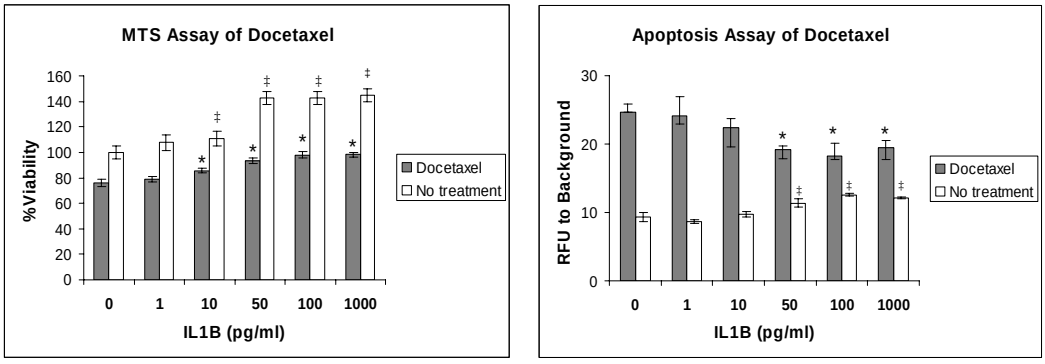
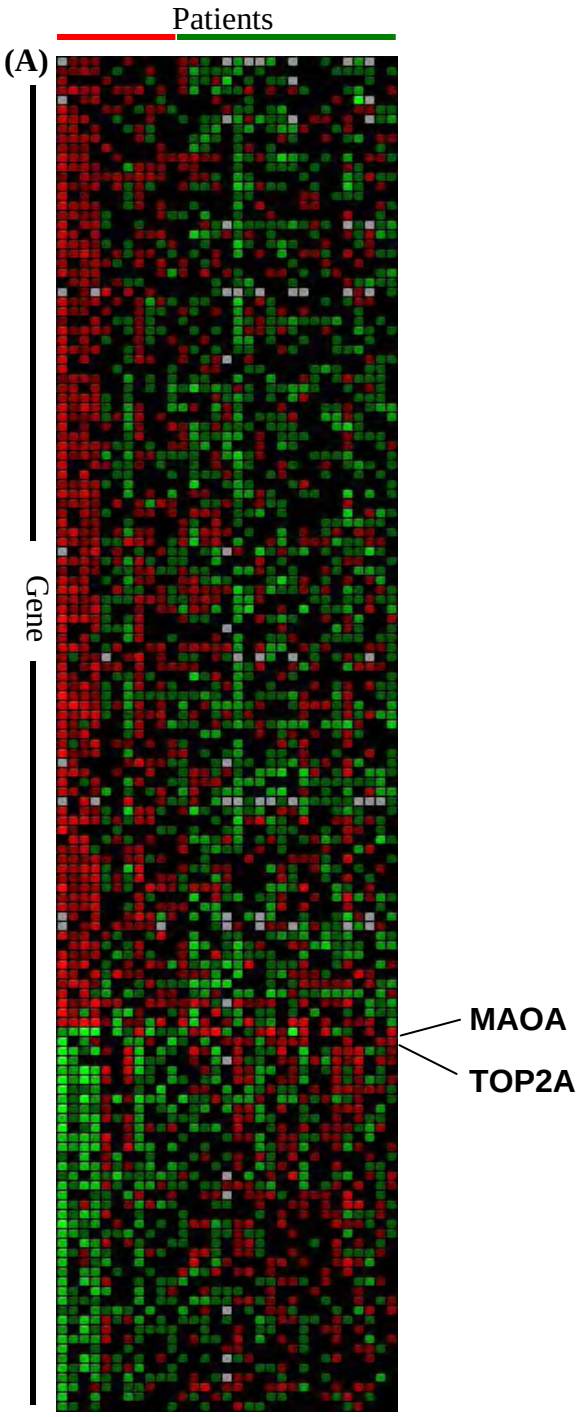
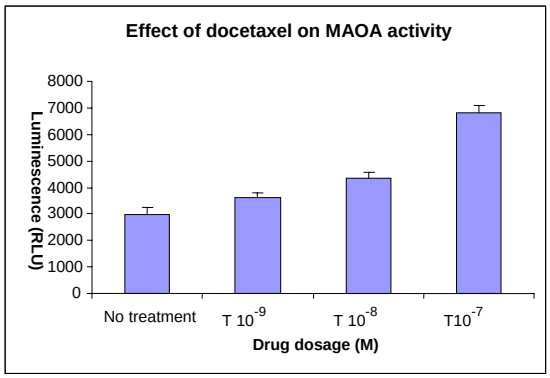
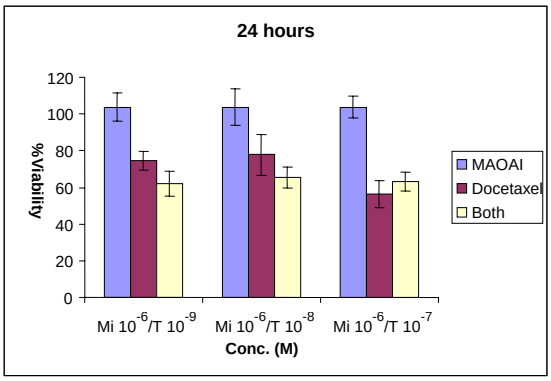
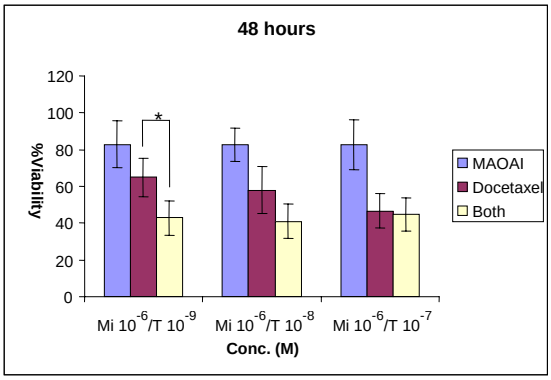


Figure 8. (A) Differentially expressed genes by survival data analysis of chemotherapy induced profile correlated with time to PSA relapse. Patients highlighted by red bar had PSA relapse and patients highlighted by green bar had no PSA relapse to date. (B) MAOA activity is induced by increasing concentrations of docetaxel. MTS cell proliferation assay of LNCAP cell. (C) 24 and (D) 48 hours after treatment with docetaxel and MAOA inhibitor. MAOA inhibitor was given one hour before administration of docetaxel into cell culture medium. %Viability was the proportion of viable cells to no treatment control cells. The concentration of MAOA inhibitor was constant through different experiments at 10^{-6}M. The concentration of docetaxel ranged from 10^{-9} to 10^{-7}M. Blue bars represent MAOA inhibitor treatment alone. Purple bars represent docetaxel treatment alone. Yellow bars represent combination treatment of MAOA inhibitor and docetaxel. (Mi: MAOA inhibitor; T: docetaxel, * p-value < 0.05 by Student t-test)


(A)
Patients
Gene
MAOA
TOP2A


(B)
Effect of docetaxel on MAOA activity
Luminescence (RLU)
8000
7000
6000
5000
4000
3000
2000
1000
0
No treatment
T 10-9
T 10-8
T10-7
Drug dosage (M)


(C)
24 hours
%Viability
120
100
80
60
40
20
0
Mi 10-6/T 10-9
Mi 10-6/T 10-8
Mi 10-6/T 10-7
Conc. (M)
MAOAI
Docetaxel
Both


(D)
48 hours
%Viability
120
100
80
60
40
20
0
*
Mi 10-6/T 10-9
Mi 10-6/T 10-8
Mi 10-6/T 10-7
Conc. (M)
MAOAI
Docetaxel
Both